THE RELATIONSHIP OF ASCORBIC ACID TO GASTRIC PHYSIOLOGY AND PATHOLOGY

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I. INTRODUCTION

A. The Discovery of the Vitamins.

A number of foods had been known to be effective as therapeutic agents for certain diseases since the eighteenth century. The eating of fresh vegetables and lemons to prevent scurvy was the result of Lind's(1) suggestion, that these foods had an antiscorbutic action. The presence of specific substances, suspected by some investigators, was confirmed by Lunin(2) in 1881. He reported that white mice could be maintained on a diet consisting of milk alone, but would soon expire on a diet of purified carbohydrate, protein, fat, minerals and water. He concluded, therefore, that some other substance or substances must have been present in the milk. Unfortunately this work did not attract much attention. Sixteen years later Eijkman(3)(4) made a very important discovery in Java. He noted that hens fed on milled rice developed a disease remarkably similar to human beri-beri. Shortly after, he cured the disease by feeding the rice bran with the milled rice. His conclusion, that the milled rice contained a toxin which was neutralized by an antidote in the bran, obscured the importance of his results until after the turn of the century. In 1911, Funk(5) succeeded in obtaining a crystalline substance from an alcoholic extract of the same bran, which would cure the diseased birds. He soon demonstrated that the new substance

was an amine and shortly after it proved successful in treating human beri-beri. It was quickly suggested that other diseases might also be caused by a lack of essential dietary substances. Funk(6), in agreement with this new theory, proposed that the new class of substances be called vitamines. After it was shown that other dietary factors were not necessarily amines, the final "e" was dropped(7).

Thus, research had begun to elucidate the nature of these essential dietary substances, to isolate them, to study their occurrence, and finally to synthesize them in the laboratory. At present there are some 15 vitamins which have been shown to exist and have been chemically identified. There are perhaps 20 more which are postulated, but as yet have not been established by chemical isolation or identification(8).

F. Vitamin C.

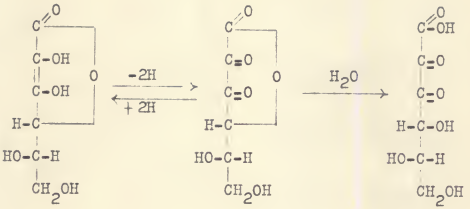
Scurvy, the typical syndrome of a vitamin C deficiency(8)(9)(10)(11), has been known among humans for many centuries and occurred epidemically during times of war, crusades, voyages and famines. During pathogenesis the intercellular substances of tissues such as bone, dentine and collagenous connective tissue appear to exist in the sol more than in the gel state.

1. Pathology of Deficiency Lesions. The significant lesions seen in acute scurvy are the hemorrhages and the skeletal defects.

a. Hemorrhagic. The hemorrhages may be found in any organ and range in size from tiny petechiae to large extravasations. On the skin they are found about the hair follicles and the sweat glands, the gingiva, and the bony prominences of the body. The deeper hemorrhages are usually found along the fascial planes in the musculature.

b. Skeletal. The skeletal lesions are found at the costochondral junctions, the distal ends of the femurs and the proximal ends of the tibias, femurs and wrists. There is usually a conical widening of the ends of the long bones. Lesions occur in the dentine layer of the teeth which seem to parallel the changes in the bone.

2. Chemistry. One hundred and seventy-five years after the appearance of Lind's(1) paper, the antiscorbutic substance in citrus fruits was isolated and identified(12)(13). The synthesis was completed by several groups of investigators(14)(15)(16)(17)(18)(19). The vitamin was shown to be a six-carbon monobasic acid of the carbohydrate series, belonging to the class of reductones, which have an ene-diol configuration(fig. 1a).



a. b. c.

FIGURE 1

The most striking property of the compound is the ease with which it is oxidized to L-dehydroascorbic acid(fig. 1b) in vivo and in vitro(20). It is believed that in solution, after oxidation, hydrolysis breaks the lactone ring. Hence, in solution some of the vitamin may occur as 2, 3-diketo-L-gulonic acid(21)(fig. 1c).

3. Physiology and Biochemistry.

a. Sources. The primates and the guinea pig, unlike other mammals, depend upon dietary sources for their vitamin C requirements. The rat is representative of a larger group of mammals which show no scorbutic symptoms when vitamin C is excluded from the diet. The vitamin is, however, found in the blood and is constantly excreted. It must be concluded that, in these animals, the vitamin has a biosynthetic origin.

(1.) Dietary. Citrus fruit, yellow and green vegetables, and tomatoes are good sources of vitamin C. Acute scurvy, at present is a medical rarity. This is probably a result of widespread knowledge of proper diet, and better techniques in food handling and preservation. Lesser ascorbic acid deficiencies, leading to sub-clinical scurvy, are, however, believed quite prevalent. These deficiencies masquerade as rheumatism, gingivitis, purpura, hemophilia and osteomyelitis. Daily allowances of ascorbic acid suggested by the Food and Nutrition Board of the National Research Council are from 30 to 75 mg. for children, 80 to 100 mg. for adolescents, 70 to 75 mg. in adults, and 100 to 150 mg. in pregnant and lactating women.

(2.) Synthesis. The exact nature of this synthesis in the rat and other mammals has remained a well-guarded secret in spite of the attempts of many competent investigators. Evidence has been presented which indicates that ascorbic acid is synthesized in isolated rat tissues in the presence of some hexose sugars. Guha and Ghosh(22) claimed that kidney, liver, heart, spleen, and leg muscle synthesized ascorbic acid in vitro, but only if mannose was present in the media. Moreover, they followed up their in vitro experiments with the injection of mannose to the living rat and reported an increased concentration of ascorbic acid in the liver(23). They concluded that mannose was a precursor in the biosynthesis. These results

apparently were confirmed by von Sztareczky(24) and Rudra(25), the latter suggesting that manganese was important in the synthesis. However, other investigators(26)(27), repeating the experiments, claimed they were not able to verify mannose as a precursor of the vitamin. On the other hand, it has been reported that glucose is utilized by tissue slices of rat intestine in the synthesis of ascorbic acid(28). Glucose, mannose, and fructose, however, are intraconvertible(fig. 2.) in mildly alkaline solution(29).

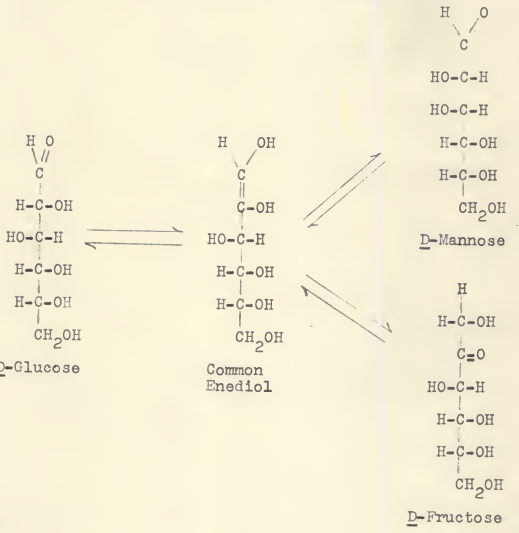
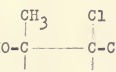


FIGURE 2

Also, it is rather well known that mannose can be utilized to increase the glycogen content of the liver. This illustrates that glucose can be easily formed from mannose with glycogen as an intermediate stage. It seems pertinent that other investigators(30) have shown that a starved rat is capable of utilizing endogenous carbohydrate, such as liver glycogen, to synthesize ascorbic acid. The tissue of starved rats contained normal amounts of ascorbic acid.

A part of the difficulty in these early experiments concerning the biosynthesis was that it was necessary to detect extremely small quantities of the vitamin. In 1940 a useful tool was discovered by Longnecker et al(31) who noted that certain barbituric acid derivatives were capable of stimulating more rapid ascorbic acid synthesis in the rat. Smythe and King(32), investigating other compounds, found that chloretone(fig. 3) was very active in



Chloretone

FIGURE 3

this regard. They reported that slices of liver, kidney or spleen from chloretone-fed rats showed definite synthetic capacity while those of a non-treated series showed dis-

tinctly less or no evidence of synthesis. It is significant that in this experiment the chloretone apparently did not supply any part of the carbon chain of ascorbic acid, since the efficiency of the synthesis was regulated only by the type of substrate present. Under these conditions the substrate found most favorable for ascorbic acid formation was not a hexose, but a mixture of the 3-carbon compounds, sodium pyruvate and glyceric aldehyde. (Hexose diphosphate was also present, as a donator of needed phosphate ion but was not incorporated into the ascorbic acid chain, since it was quantitatively recovered.) A substrate of mannose, glucose and ketogulonic acid was ineffective. Thus, it appears that under chloretone stimulation *in vitro* the ascorbic-acid carbon chain is formed by condensation of two three-carbon fragments.

In some *in vivo* studies, however, Jackel *et al*(33) administered glucose, uniformly labeled with carbon-14, to chloretone stimulated rats and recovered from the urine a small but definite amount of uniformly labeled ascorbic acid. These results posed the question as to whether the administered glucose had undergone glycolysis together with rearrangement of the resulting triose phosphates to give ascorbic acid, or if the carbon chain of the glucose was utilized in its intact form. Horowitz *et al*(34) soon demonstrated that the administration of D-glucose-1-C^{14} to rats led to the isolation of L-ascorbic acid-6-C^{14} in the urine.

Similarly, he later reported(35) that the administration of D-glucose-6-C^{14} resulted in the isolation of L-ascorbic acid-1-C^{14}. This shows quite definitely that there is no glycolysis or rearrangement of the glucose carbon chain during its conversion to ascorbic acid.

Conclusions based on the increased rate of ascorbic acid synthesis brought about by the administration of chloretone may not, of course, be applicable to the normal biosynthetic mechanism. It is conceivable, however, that chloretone acts similarly to some naturally occurring compound in accelerating the production of ascorbic acid.

b. Metabolism. Despite our long standing knowledge of the nature of vitamin C, surprisingly little is known about the fundamental metabolic role which it plays in the organism.

(1.) Absorption, transport, and localization. Dietary ascorbic acid is absorbed through the small intestine in the guinea pig(36) and is transported in the blood. Varying concentrations of the vitamin have been found in a variety of tissues, the adrenal having the highest concentration. The adrenal has been considered as a storage depot for ascorbic acid but this cannot be considered entirely correct, since one cannot deplete the guinea pig adrenal of all its ascorbic acid, even in scurvy, nor can the level in the adrenal be raised dramatically by feeding excess vitamin C(37). Considerable amounts are found in the

gastrointestinal tissue, the mucosa of humans having a greater concentration than the submucosa or muscular layers (38).

(2.) The oxidation and reduction of ascorbic acid *in vivo*. L-Ascorbic acid exists in the body in two forms, both of which exhibit antiscorbutic properties. It is found in tissue predominantly in the reduced form (fig. 1a). Only in the urine is a considerable amount of the oxidized form, dehydroascorbic acid (fig. 1b) observed. It had long been supposed that the oxidized form was reduced, perhaps enzymatically, in the blood stream, but Borsook *et al*(20) proved that this was not correct. They demonstrated conclusively that the reduction of some portion of the dehydroascorbic acid took place in the tissues and not in the blood. In addition they postulated that the reducing agent was glutathione. Furthermore, these investigators suggested that some of the oxidized form, dehydroascorbic acid, is constantly being hydrolyzed to 2, 3-diketo-L-gulonic acid and finally oxidatively fragmented to oxalic acid and L-threonic acid(fig. 4).

(3.) The formation of intercellular substance. It is agreed that vitamin C influences the formation of intercellular substances in bone, dentine, and collagenous connective tissue. The mechanism by which this deposition is accomplished, however, is not completely understood. Two theories have been advanced which attempt an

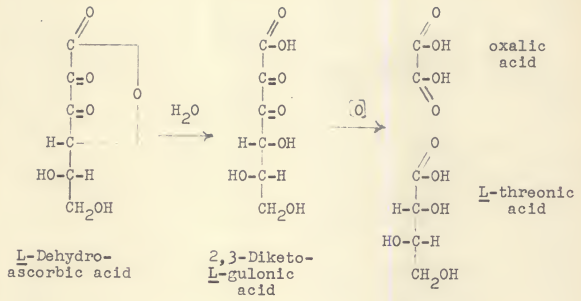


FIGURE 4

explanation. Wolbach and Howe(39) believe that in C-deprivation, the cells of some tissues form fluids rather than their usual denser products, dentine, bone and collagen. The vitamin is considered to be an essential ingredient in the intercellular substances. The Höjer(40) theory proceeds from the assumption that ascorbic acid depletion causes an atrophy of skeletal cells, that the fluid formed is not a product of the cells, and that the vitamin is not an integral part of the normal intercellular substance. Although both theories were warmly defended, little experimental work was done which substantiated either until recently when Burns et al(41) administered tagged ascorbic acid to guinea pigs. The animals were kept on a C-free

diet for two weeks and then given an intraperitoneal injection of 1-C^{14}-L-ascorbic acid. After 24 hours considerable activity was present in the skin and the nasal septum. After isolation of collagen, chondroitin-sulfuric acid, and glucuronic acid from these tissues, however, no activity was detectable. All of the activity in the septum was shown to be due to radioactive ascorbic acid, but the activity in the skin was not identified. Unless ascorbic acid was incorporated into a poly-saccharide chain in the skin, which was removed in extracting the components, it can be said that the vitamin is not incorporated into the extracted intercellular substances of skin or cartilage. This may be interpreted as substantiating Höjer's theory. Wolfer and his associates(42) have reported the effect of ascorbic acid depletion on the healing of wounds. These investigators used human subjects, and demonstrated that in vitamin C depletion, wounds show a 50% decrease in tensile strength and a lack of collagen and reticulum in the new tissue. The wounds also require a longer time for complete healing.

(4.) Relationships to carbohydrate metabolism. A relationship between ascorbic acid and carbohydrate metabolism has been reported by Sigal and King (43) who demonstrated that the capacity of the guinea pig for metabolizing glucose rose moderately in the prescorbutic and scorbutic states of a vitamin C deficiency. Administra-

tion of ascorbic acid has been shown to increase liver glycogen in rabbits(44). Although the picture is not entirely clear, it seems evident that ascorbic acid takes part in regulating carbohydrate metabolism.

(5.) Suggested respiratory enzyme function. Since ascorbic acid has two labile hydrogen atoms, and is reversibly oxidizable, its participation in some respiratory enzyme system has often been suggested. Szent-Györgyi(45) postulated an elaborate scheme of a hydrogen transfer mechanism which included the cytochrome C-cytochrome oxidase system and ascorbic acid. Stotz et al (46), confirmed the in vitro oxidation of ascorbic acid by the cytochrome C-cytochrome oxidase system, but feel that there is not enough evidence for ascorbic acid as a respiratory agent to warrant its inclusion in a respiratory system.

(6.) The excretion of ascorbic acid and its metabolites. The vitamin is excreted for the most part in the urine(47) but also to a minute degree in the sweat (48) and feces(49). Burns et al(41) have recently confirmed the designation of oxalic acid as a metabolite in the guinea pig with the use of 1-C^{14}-L-ascorbic acid. Over a period of 10 days they recovered 90% of the radioactivity of an injected dose of the tagged vitamin, and showed that approximately 25% of the recovered radioactivity was present in the urine as oxalic acid, while 75% was expired as carbon dioxide.

4. Gastrointestinal Relationships.

a. Occurrence of ascorbic acid in the tract. Relatively few reports have appeared regarding the level of vitamin C in the mammalian gastrointestinal tract, however, the available evidence indicates that the vitamin is present in these tissues and the gastric juice.

(1.) The gastric juice. Determination of ascorbic acid in gastric juice has been carried out by Demole and Issler(50) who found a range of 0.15 to 1.50 milligrams percent in a series of human subjects. Peters and Martin(51) conducted a similar study and reported a slightly lower range, varying from 0.046 to 1.04 milligrams percent. These investigators also reported the ascorbic acid content of canine gastric juice as ranging from 0.33 to 1.51 milligrams percent. The gastric juice in both of these studies was taken from fasted stomachs, thus the possibility of dietary ascorbic acid in the gastric juice was eliminated. The ascorbic acid in the juice may have arisen from gastric secretion, salivary secretion or possibly duodenal regurgitation. The latter possibility is remote, however, since regurgitation is not considered strictly physiological, and ascorbic acid was found in every sample of gastric juice. Furthermore, Reid(52) has demonstrated that a considerable amount of ascorbic acid can be found in the gastrointestinal contents within four hours after an intraperitoneal injection of the vitamin to

C-deficient guinea pigs. This finding supports the case for gastrointestinal secretion of ascorbic acid.

(2.) The gastric wall. Considerable variation is found in the reports of determinations of ascorbic acid in human gastric tissue. Ludany and Zselyonka(53) indicate that the average gastric mucosa concentration is approximately 25 milligrams percent, the pyloric tissue containing slightly less than the fundic tissue. Other investigators(54) have claimed the average concentration to be as low as 1 to 3 milligrams percent. Such wide variation is probably not physiological, but more likely introduced in determination. The lower values may have been caused by negative errors, easily introduced by the rapid and irreversible oxidation of the vitamin in autolyzing tissue. Moreover, the ascorbic acid was determined after being homogenized with trichloracetic acid and sand. This method exposes the ascorbic acid to enough air to cause considerable oxidative degradation, and also to traces of copper which catalyze the oxidation.

Ludany and Zselyonka(53) reported, also, that the concentration of the vitamin was highest in the small intestine and declined regularly, superior and inferior to that point. They also pointed out that the layers of the gastrointestinal tract, mucosa, submucosa and muscle, have ascorbic acid concentrations which descend in that order.

Peters and Martin(51) have determined the concentration of vitamin C in the gastric tissue of the dog. In a study which included only two dogs they found pyloric values of 0.55 and 3.72 milligrams percent, while in the fundus the values obtained were 5.3 and 5.7 milligrams percent. Since sand-mortar homogenates were employed here also, some ascorbic acid may have been destroyed.

Unfortunately, no values appear in the literature for the gastric tissue of the rat and guinea pig. Moreover, the stomach has not been eliminated as a site of ascorbic acid synthesis in the rat. These phases of gastric physiology have been pursued in present investigation.

b. The secretion of gastric juice.

(1.) Histamine. The oxidative decarboxylation of the amino acid histidine yields histamine, which is responsible, in part, for stimulating gastric secretion(55). The activity of histamine is destroyed through the action of an enzyme, histaminase, which can be isolated from kidney tissue(56). Small amounts of ascorbic acid have been shown to act as an activator for the enzymatic destruction of histamine *in vitro*, but larger amounts caused inhibition of the enzyme, which resulted in slower destruction of histamine(57). On the other hand, Leone and Crimaldi(58), pursuing the problem *in vivo*, claimed that C-deficient guinea pigs showed increased histamine blood levels. If this C-

deficiency was characterized by a sufficiently low ascorbic acid titer in the blood, one would have expected, in the light of the in vitro investigation(57) that the histamine blood level would have decreased. Further experiments involving the actual hydrochloric acid secretion, initiated by histamine, tended to clarify the conflicting ideas.

(2.) Hydrochloric acid. The experiments of Caffe and Dulce(59) have shown that the intravenous injection of sodium ascorbate to humans results in an increase in free and total hydrochloric acid as well as an increased volume of gastric juice. These results which have been confirmed by Lucksch(60) and Nicolesco et al(61), lend force to the theory that the level of blood ascorbic acid is proportionally related to the amount histamine in the blood and, hence, to the rate of gastric acid secretion. It seems evident that additional valuable information could be obtained if gastric secretion was collected from C-deficient guinea pigs while simultaneously administering ascorbic acid. This investigation includes experiments of this type.

c. The motility of the stomach. The peristaltic motion of the stomach is quite necessary to assure adequate mixing of the contents with the gastric juice. Tudoranu and Dimitrui(62) have carried out experiments on humans which suggest that the duodenal absorption of the

ascorbic acid causes an initial retardation and a subsequent increase in the motility of the stomach.

d. Gastric proteolysis. Since ascorbic acid is present in the gastric juice the possibility of some action upon peptic proteolysis suggested itself. Vitamin C has been claimed to increase peptic activity in vitro(63) from 10 to 25 percent. This action must, of course, be distinct from action on hydrochloric acid secretion, since in the in vitro experiment the hydrochloric acid concentration is constant. The need for further investigation of the role of ascorbic acid in peptic proteolysis, directing emphasis toward the oxidation of ascorbic acid, has been apparent for some time. A study of this type is a part of the present investigation.

e. Gastric disease. The presence of ascorbic acid in the tissue and the secretion of the stomach has directed attention toward the role of this vitamin in human gastric pathology. Such disorders as achlorhydria, ulcers, and gastric carcinoma may be reflected in abnormalities in the vitamin C metabolism of the stomach itself. Einhauser (64) has suggested that a reciprocal effect is also a possibility. He states that gastric disease, notably gastroenteritis, may impair intestinal absorption and decrease the amount of ascorbic acid which enters the systemic circulation. In this case a deficiency of the vitamin could be the result of gastrointestinal disease, while in other instances a

deficiency may well be a contributing cause of stomach disorder.

(1.) Achlorhydria and hypochlorhydria. Blood ascorbic acid(65) and gastric juice ascorbic acid(51) have been shown to be subnormal in patients suffering from achlorhydria. In these cases, it is improbable that the decreased amount of available ascorbic acid completely prevented the production of hydrochloric acid, nevertheless, it seems more than coincidental that the achlorhydria appeared to accompany the low ascorbic acid blood levels. Singer(66) showed that of 25 patients having gastric disease, 21 showed decreased ascorbic acid in the blood. The diagnoses of these patients indicated cases of gastritis, and gastric and duodenal ulcers accompanied by varying degrees of hypo- and hyperacidity. Again, adhering to the theory that the blood level of ascorbic acid is proportionately related to the amount of histamine present and hence, directly related to hydrochloric acid production, the occurrence of hyperchlorhydria in the presence of low ascorbic acid blood levels seems contradictory. A reasonable explanation, however, stems from the fact that gastric secretion can also be stimulated through the vagus nerve, a mechanism which is apparently separate from the histamine pathway.

(2.) Ulcers. Hanke(67) and later Smith and McConkey(68) examined the gastrointestinal tract of C-deficient guinea pigs and reported that 26 percent of

the animals had ulcers of the gastric, pyloric or duodenal variety. The latter authors also demonstrated that mechanical injury to the gastric mucosa of guinea pigs maintained on an adequate diet resulted in rapid healing. Identical injury to the mucosa of C-deficient animals, however, resulted in ulcers. The authors conclude that hypovitaminosis C lowers the resistance of the mucosa and reduces its capacity for regeneration. The administration of cinchophen to dogs is also known to induce gastric ulceration. When large doses of ascorbic acid were administered with the cinchophen, the ulceration was prevented in greater than 60 percent of the cases(69). The overdosage of ascorbic acid should be suspected of having induced more rapid hydrochloric acid secretion in the stomach, which would not, at first glance, seem conducive to the healing of the cinchophen-induced ulcers. However, the action of ascorbic acid in accelerating regeneration at the site of wounds apparently plays a primary role in this instance. The work of Iacobovici et al (54) supports the contention that ascorbic acid is active in ulcer regeneration areas. They determined ascorbic acid in the tissue at gastric ulcer sites in humans and demonstrated that the regenerative or actively dividing area of the ulcer contained larger amounts of ascorbic acid than other locations.

The gastric ulcers discovered in human patients are usually associated with hyperacidity in the stomach.

The excessive hydrochloric acid which may in itself induce ulcers(70) is believed to be brought about through vagal control of secretion. Some reports indicate that ulcer patients have a lower concentration of ascorbic acid in their blood than is normally found(71)(72). It may be that ascorbic acid, in the process of activating histaminase, is destroyed. Such a hypothesis would account for the decreased ascorbic acid levels, as well as the hyperacidity, in many ulcer patients. Schultzer(73), however, claims that the low blood level of vitamin C is due to the low C diet which ulcer patients receive. This claim may be partly valid, however, interpretations of the existing experimental and clinical evidence suggest that more than dietary insufficiency is involved.

(3.) Gastric adenocarcinoma. Minor and Ramirez(74) have measured the daily utilization of vitamin C in patients with malignant and non-malignant diseases. They fed large daily doses of ascorbic acid until the daily urinary excretion became constant. The difference between the amount administered and the amount recovered in a 24 four hour period represented the daily utilization. Their results show that the malignant cases utilized more ascorbic acid than the non-malignant cases. Even more significant, however, is the fact that two cases of gastric adenocarcinoma utilized far more ascorbic acid than either the remaining malignant or non-malignant cases.

Similarly, it is probably significant that Leise et al(75) have reported that the percentage of takes in transplantation of a hepatoma is increased from 36% to 80% in C57L(Fx) mice when supplementary ascorbic acid is supplied together with rutin. Here again, as in the regenerative tissue of ulcers, is suggestive evidence that actively dividing cells require larger quantities of ascorbic acid.

The investigation of gastric adenocarcinoma is difficult, since spontaneous occurrence of this form of cancer in experimental animals is rare. In a comprehensive survey of the literature Horn and Stewart(76) have found only 3 such cases of adenocarcinoma of the stomach in mice. Moreover, there is as yet, no convenient, reproducible method for its induction(77). Most of the attempts at gastric tumorigenesis have employed mice or rats, but the guinea pig, which has a vitamin C metabolism similar to man, might prove more susceptible. Russell et al(78) injected 20-methylcholanthrene into the axillary subcutaneous tissue of guinea pigs which had been divided into 3 groups. Ascorbic acid was excluded from the diet of Group 1 for 14 days, a period during which the animals lost weight and developed definite scorbutic symptoms. Lettuce was then fed with the diet to supply ascorbic acid until the animals had regained the lost weight, when the lettuce was removed and the cycle repeated. This sequence was repeated through out the experiment. In Group 2, lettuce was constantly

available. The diet of Group 3 was restricted in amount so that a loss in weight, similar to that seen in the first group, would occur. The animals in this group were supplied with adequate ascorbic acid by subcutaneous injection. Sarcomas developed in all groups, the incidence apparently unrelated to the difference in treatment but tumors appeared significantly earlier in Group 1(the deficient group) than in the control, Group 2, in which lettuce was always available. There was apparently no significant difference in the time of induction of the tumors of Group 1 and Group 3 (the inanition group). There was also no significant difference between Groups 2 and 3. This seems to lead to the conclusion that a significant difference occurred only when there was a simultaneous loss of weight, deficiency of vitamin C, and a deficiency of the other factors contained in lettuce.

The carcinogenicity of 2-aminofluorene, which induces a wide variety of tumors in rats, has not been determined in the guinea pig. In the light of the existing relationships between ascorbic acid and the stomach, in the present study, it seemed advantageous to determine (a) the carcinogenicity of 2-aminofluorene for the guinea pig and (b) if a relatively constant, but decreased intake of ascorbic acid, simulating a sub-clinical deficiency, together with orally administered 2-aminofluorene, would alter the type or rate of tumorigenesis. Incorporation of the carcino-

gen into the diet assured a continuous passage of the compound through the stomach, the target organ. A simultaneous ascorbic acid deficiency was expected to initiate gastric dyscrasia favorable to the induction of tumors by the carcinogen.

(4.) Gastritis. Because it has been suspected that gastritis is often a precursor of cancer(79), a study of the relationship between gastric ascorbic acid and gastritis in both the rat and the guinea pig has been undertaken in the present investigation.

II. STATEMENT OF THE PROBLEM

The evidence presented in Section I indicates that some physiological and pathological processes occurring in the gastro-intestinal tract are influenced by ascorbic acid. The mechanisms of these actions, however, are not clear. The following investigations, therefore, were undertaken.

A. The Effect of Ascorbic Acid on Gastric Proteolysis.

The presence of ascorbic acid in gastric juice suggested the possibility of a relationship between this vitamin and gastric digestion. This hypothesis was investigated, directing emphasis toward observations on the oxidation of ascorbic acid, since it is easily oxidized in vivo and in vitro.

B. An Investigation of Possible Gastric Synthesis of Ascorbic Acid in the Rat.

Since the stomach had not been eliminated as a site of synthesis of ascorbic acid in the rat, while several other tissues have been shown to be active in this respect, it seemed necessary to study this possibility.

C. A Study of Ascorbic Acid in Gastric Tissue of the Normal and Gastritic Rat.

In the light of existing, yet relatively unclear, relationships between ascorbic acid and some types of gastric disease in man, the need for additional factual information was apparent. In the rat, in which abundant biosynthetic

ascorbic acid is available, glandular gastric disorder is uncommon. The ability to induce a gastritis, (perhaps the only readily reproducible example of a gastric glandular dyscrasia) presented an opportunity to investigate a common type of gastric disease in its relationship to ascorbic acid under well-controlled conditions.

D. A Study of Ascorbic Acid in Gastric Tissue of the Normal and Gastritic Guinea Pig.

In order to effect a comparison of gastritis-ascorbic acid relationships in the rat to those in a species having similar vitamin C requirements to man, experiments identical to those described in Section II C were undertaken with the guinea pig.

E. The Relationship of Ascorbic Acid to Gastric Secretion in Guinea Pigs.

The effect of ascorbic acid on gastric secretion is not completely understood in spite of the interest of many investigators. One probable reason may be that in many experiments dogs were employed. The dog suffers the same experimental limitation as the rat, in that ascorbic acid is biosynthetically supplied. It is, therefore, impossible to observe the effects of a C-deficiency on canine gastric secretion. The present experiments employed the guinea pig, in which a deficiency of vitamin C can be induced.

F. The Effects of Ascorbic Acid on Tumor Induction in Guinea Pigs by Feeding a Carcinogen.

It has been reported that an ascorbic acid deficiency aids in the initiation of various pathological conditions in the gastro-intestinal tract. Moreover, gastric adenocarcinoma is rare in laboratory animals, which, with the solitary exceptions of the primates and the guinea pig, have abundant biosynthetic ascorbic acid. It seemed advisable, therefore, to determine if a C deficiency would induce gastric dyscrasia favorable to the induction of stomach tumors in guinea pigs by the oral administration of a known carcinogen.

III. EXPERIMENTAL METHODS

The study of the effect of ascorbic acid on gastric proteolysis, A, and the investigation of the possible gastric synthesis of ascorbic acid in the rat, B, were conducted in vitro.

A. The Effect of Ascorbic Acid on Gastric Proteolysis.

Digestion mixtures were prepared by placing 0.1 - 1.0 g. crude pepsin* or 72 mg. crystalline pepsin together with 1.0 g. casein** and 200 ml. distilled water in a 400 ml. Erlenmyer flask. The pH of the mixture was adjusted to 2.0 with concentrated hydrochloric acid using a Beckman Model G pH meter. The flask was stoppered with a gauze plug and placed in a constant temperature bath at 37.6° C. Determinations of the pH during the digestion indicated no change in the hydrogen ion concentration, thus no other buffer was employed. To certain of these digestions varying amounts of ascorbic acid were added.

The progress of the casein proteolysis was followed by periodically analyzing aliquots of the digestion mixture for amino nitrogen by the Van Slyke method(80). This technique consists essentially of measuring the volume of gaseous

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* Pepsin, U.S.P., granular, P-54. Fisher Scientific Co.

** Casein, soluble, C-202. Fisher Scientific Company

nitrogen evolved when the primary amino groups decompose in the presence of nitrous acid. Another gas, nitric oxide, is formed but it is absorbed in alkaline potassium permanganate, thus allowing the measurement of only the nitrogen.

The concentration of ascorbic acid was followed by analyzing aliquots from the digestion mixture regularly. The method chosen for the determination of ascorbic acid was that of Roe et al(21)(81). Essentially, their procedure consists of oxidizing ascorbic acid to 2, 3-diketo-L-gulonic acid and coupling at the 2 and 3 position with 2, 4-dinitrophenylhydrazine, thus forming the osazone. The optical density of this red compound (maximum: 540 millimicrons) is measured in acid solution in a colorimeter. In the present study, however, a Beckman Model DU Spectrophotometer was employed. Using this method the amount of oxidized ascorbic acid (dehydro-ascorbic acid and diketogulonic acid) as well as the amount of total ascorbic acid (dehydroascorbic acid, diketogulonic acid, plus ascorbic acid) was determined in a given sample. The amount in the reduced form (ascorbic acid) was taken as the difference between the total and oxidized ascorbic acid.

Anaerobic digestions were conducted by bubbling nitrogen through the digestion mixture. Table 1 summarizes the experimental conditions.

B. The Investigation of Possible Gastric Synthesis of Ascorbic Acid in the Rat.

TABLE 1

Experimental Conditions of Peptic Proteolysis

DIGESTION NO.	AIR	PEPSIN (crude) g.	PEPSIN (crystal.)	ASCORBIC ACID ADDED AT 15 min. mg.	ASCORBIC ACID ADDED AT 120 min. mg.
1	adm*	1.0	0	0	0
2	"	1.0	0	100	0
3	"	1.0	0	100	50
4	exc**	1.0	0	0	0
5	"	1.0	0	100	0
6	adm	0.1	0	0	0
7	"	0.1	0	10	0
8	"	0	72***	0	0
9	"	0	72****	0	0
10	"	0	72***	10	0
11	"	0	72***	10	0
12	exc	0	72***	10	0

* air admitted

** air excluded

*** crystalline pepsin prepared(82) from crude material obtained from Fisher Scientific Company.

**** crystalline pepsin obtained from General Biochemicals Inc.

The stomachs of 3 Sprague-Dawley-Holtzman male rats weighing 300-350 g. were used in this experiment. In each case the animal was sacrificed by neck stroke and the body cavity opened. A straight needle, threaded with silk thread, was passed through both gastric walls transversely at the pyloric sphincter. The ends of the thread were tied, forming a loop. The same procedure was repeated at the base of the esophagus. The stomach was then freed from the remainder of the tract by cutting superior to the esophageal loop and inferior to the pyloric loop, and from adhering connective tissue of the omentum and pancreas. The inner, mucosal surface of the stomach was rinsed well with Ringer-Tyrode solution. Using the thread loops, the excised stomach was suspended in 60 ml. Ringer-Tyrode solution at a constant temperature of 37° C. and supplied with oxygen (fig. 5). Aliquots of the sustaining solution were removed at intervals and analyzed quantitatively for ascorbic acid(21). The experiments differed from one another in the following manner:

Experiment 1: Incubation of the stomach of a rat which had been fasted for 48 hours.

Experiment 2: Incubation of the stomach of a rat which had been on a C-deficient diet for 48 hours.

Experiment 3: Incubation of the stomach of a non-fasted rat which, after rinsing with Ringer-Tyrode solution, had been filled with glass beads.

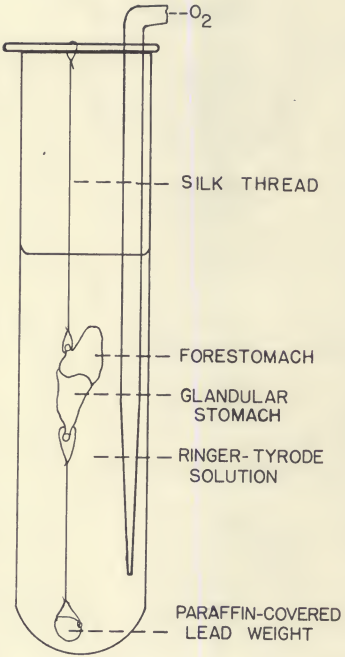

FIGURE 5

APPARATUS FOR IN VITRO INCUBATION OF RAT STOMACH

C. A Study of Ascorbic Acid in Gastric Tissue of the Normal and Gastritic Rat.

Twenty-seven male Sprague-Dawley-Holtzman rats, weighing 200-250 g. and receiving Purina Laboratory Chow and tap water ad libitum, were used in this study. Eugenol (4-allyl-2-methoxyphenol), which has been shown to produce gastritis in rats(83), was administered daily as an aqueous 1.0% emulsion in 3 ml. doses for 7 days. Control groups were given the same volume of distilled water daily. The rats were divided into groups according to the manner in which the eugenol emulsion or the distilled water was introduced into the gastrointestinal tract.

Group 1: Distilled water was administered by stomach tube.

Group 2: Eugenol emulsion was administered by stomach tube.

Group 3: The rats were subjected to (a) light ether anesthesia, (b) a 2-3 cm. abdominal incision, and (c) injection of eugenol emulsion into the upper duodenal lumen. The musculature and skin were sutured with silk thread. This operative procedure was repeated daily for 7 days.

Group 4: The rats were subjected to the same daily operative procedure as those of Group 3, but eugenol emulsion was injected into

Group 4: (continued)

the gastric lumen.

On the eighth day the animals were sacrificed by neck stroke. The forestomach, glandular stomach, and adrenals were observed grossly, excised, weighed and subjected to analysis for total and oxidized ascorbic acid(81).

D. A Study of Ascorbic Acid in Gastric Tissue of the Normal and Gastritic Guinea Pig.

Twenty-one male Rockland Farms guinea pigs weighing 350-450 g. were used in this study. They received Purina Rabbit Chow Checkers*, and tap water ad libitum and daily intramuscular injections containing 25 mg. sodium ascorbate in 1.0 ml. physiological saline. The guinea pigs were divided into 2 groups. Eugenol as a 1.0% aqueous emulsion, Group 1, or distilled water, Group 2, was administered daily by stomach tube in 3 ml. doses for 7 days. On the eighth day the animals were sacrificed by neck stroke. The stomach and adrenals were observed grossly, excised, weighed, and subjected to analysis for total and oxidized ascorbic acid (81).

E. The Relationship of Ascorbic Acid to Gastric Secretion in Guinea Pigs.

1. Operative procedure. Eight male Rockland Farms

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* preliminary analyses of the complete stock of Purina Rabbit Chow Checkers used in this entire study indicated that the concentration of ascorbic acid was less than 0.08 mg. %.

guinea pigs weighing 350-450 g. were employed in this series of experiments. After a 24 hour fasting period, each animal was subjected to surgery to introduce a gastro-cutaneous fistula, for the purpose of collecting gastric juice. The animal was partly anesthetized by intraperitoneal injection of 15.0 mg. Nembutal/ Kg. of body weight. Further anesthesia* was induced by administering ether with an inhalation cone. The abdominal hair was removed with electric clippers, the skin swabbed with 70% ethyl alcohol, and a 3 cm. midline, abdominal incision made at the level of the stomach. The stomach was exposed and a nylon thread securely sutured in the anterior wall at a point just superior to the pyloric sphincter. A perforation 3-4 mm. in diameter was made in the musculature and skin to the right of the midline incision with a blunt probe. The thread was drawn through this perforation until a 4 mm. "bubble" of the stomach protruded through the abdominal wall. One suture was introduced which secured the exteriorized stomach tissue to the abdominal wall. The original incision was closed. The wall of the protruding area of the stomach was perforated and the area of attachment of the thread excised with a scissors. Six individual nylon sutures were placed so as to hold the

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* preliminary anesthesia trials indicated that complete anesthesia with Nembutal required a dose which also induced a deep state of shock. The use of ether together with the lower dose of Nembutal circumvented this difficulty.

prepared apertures in the stomach and body wall in apposition. No gastric juice was collected from any fistula animal until the 7th day following the operation.

2. Diet and ascorbic acid control. Only milk was allowed on the day following the operation. On the next day, and succeeding days, except during pre-collection fasting periods, Purina Laboratory Chow Checkers(see footnote, p. 34) and tap water were available. Also on the 2nd through 7th days following the operation all guinea pigs received 13.8 mg. sodium ascorbate*/ Kg. body weight intramuscularly in physiological saline. At the end of the 7th day the animals were divided into 2 groups of 4 each.

Group 1: The frequency of injection of sodium ascorbate was reduced to 1 per week at the same dosage and administered more than 48 hours previous to gastric juice collection.

Group 2: The injections of sodium ascorbate were continued at the same dosage and frequency at which they were begun.

3. Gastric juice collections. Collections of gastric juice were made 1-2 times per week on each animal. The

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* This administration of sodium ascorbate is equivalent to that given by Keuther et al(84) as the daily requirement for the guinea pig.

day previous to collection, fresh milk was the only food allowed. On the day of collection, the animal was placed in a restraining device(fig. 6). A glass cannula was introduced into the gastric lumen by way of the fistula. The residual gastric juice which flowed out was collected for 4-5 minutes in a 15 ml. graduated centrifuge tube. The stomach cavity was then washed 3 times with 2 ml. portions of physiological saline. When drainage of the saline appeared nearly complete, collection of gastric juice for consecutive 5 or 10 minute periods was begun. Saline, ascorbic acid in saline, or histamine diphosphate in saline was injected intraperitoneally at various times during the collection period, which averaged about 75 minutes. A series of 4-5 75 minute collections were obtained from each animal, but in no case were more than 2 collections per week permitted.

4. Analyses for total gastric acid and ascorbic acid in gastric juice. The volume of secretion for each collection interval was recorded. This volume or an aliquot was titrated against standardized 0.001N sodium hydroxide using phenolphthalein as an indicator. In some cases the ascorbic acid concentration of the gastric juice was determined(81).

F. The Effect of Ascorbic Acid on Tumor Induction in Guinea Pigs by Feeding 2-Aminofluorene.

A total of 48 male Rockland Farms guinea pigs, having an average weight of 370 g. at the start of the experi-

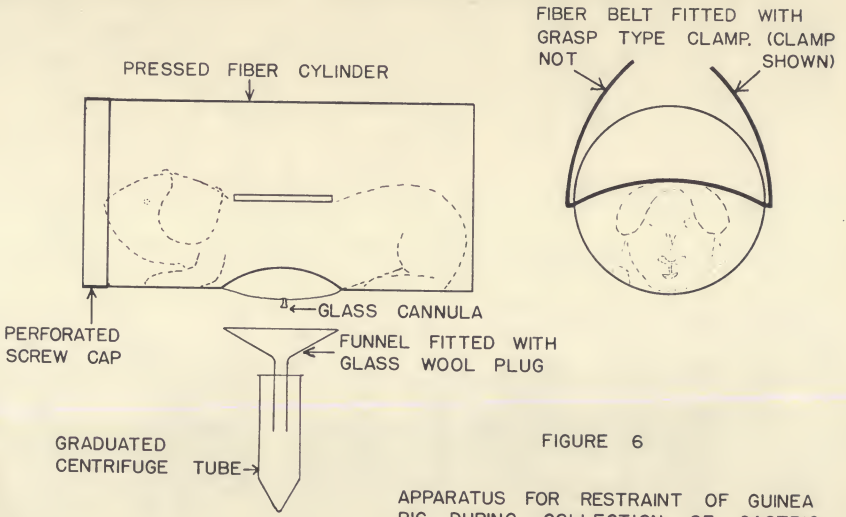

FIGURE 6

APPARATUS FOR RESTRAINT OF GUINEA PIG DURING COLLECTION OF GASTRIC JUICE

ment, were housed in pairs in an air-conditioned room in which the temperature was between 78 and 80° F. constantly. A freshly prepared, aqueous, neutralized solution of ascorbic acid, having a concentration of 70 mg. / ml., was fed directly into the mouths of all guinea pigs twice weekly from a 2 ml. tuberculin syringe equipped with a 22 gauge, blunt needle. The treatment of the animals, which were divided into 4 groups of 12 each, differed only in the volume of the ascorbic acid solution and the composition of the diet which was administered. These differences are summarized in Table 2. The animals were weighed 2-4 times

TABLE 2

Conditions of Administration of Ascorbic Acid and 2-Aminofluorene to Guinea Pigs.

GROUP	BASAL DIET OFFERED (ad libitum)	ORALLY ADMINISTERED ASCORBIC ACID mg./100 g. body weight/week
1.	H_2O & PRCC*	17.0
2.	H_2O & PRCC with 0.06% AF**	17.0
3.	H_2O & PRCC	2.8
4.	H_2O & PRCC with 0.06% AF	2.8

* Purina Rabbit Chow Checkers, Purina Mills, St. Louis, Mo. (see footnote, p. 34)

** 2-aminofluorene, m.p. 126-127.5°C. Prepared according to the method given in Organic Syntheses(85).

monthly throughout the experiment and examined weekly for palpable tumors.

1. Preparation of ascorbic acid solution for oral administration. A 700 mg. portion of L-ascorbic acid was dissolved in approximately 3 ml. distilled water and quantitatively transferred to a 10 ml. volumetric flask. A 4.5 ml. portion of aqueous 7.6% sodium bicarbonate was added to the solution. The flask was filled to the 10 ml. mark after most of the carbon dioxide had been given off.

2. Impregnation of the basal diet with 2-aminofluorene. An 8118 g. portion of Purina Rabbit Chow Checkers was mixed with 4.86 mg. 2-aminofluorene in 1500 ml. acetone. The wet food was spread on a stainless steel tray and allowed to dry for at least 4 days.

IV. RESULTS AND DISCUSSION

A. The Effect of Ascorbic Acid on Gastric Proteolysis.

The data obtained in these experiments has been embodied in the graphs of figures 7 through 11. Time in hours (horizontal) has been plotted against proteolytic activity in ml. of nitrogen(left vertical) and the ratio of oxidized to reduced ascorbic acid(right vertical). The numbered curves trace the proteolytic activity during time, while those bearing the corresponding number, followed by a letter, trace the ascorbic acid ratio during the same digestion. The time of addition of ascorbic acid is indicated by a short, vertical, dotted line intersecting the base line.

Example: Curve 2, figure 7 traces the progress of digestion 2 during approximately 7 hours, while curve 2A follows the ratio of oxidized to reduced ascorbic acid in the same digestion.

1. Crude pepsin experiments. The typical control digestion of casein using crude pepsin is shown in curve 1, figure 7. The addition of ascorbic acid resulted in a decreased proteolytic rate, over the first 2½ hours, which was followed by a rather sharp acceleration, curve 2. The addition of more ascorbic acid extended the period of decreased rate, shown in curve 3. The ratio of oxidized to reduced

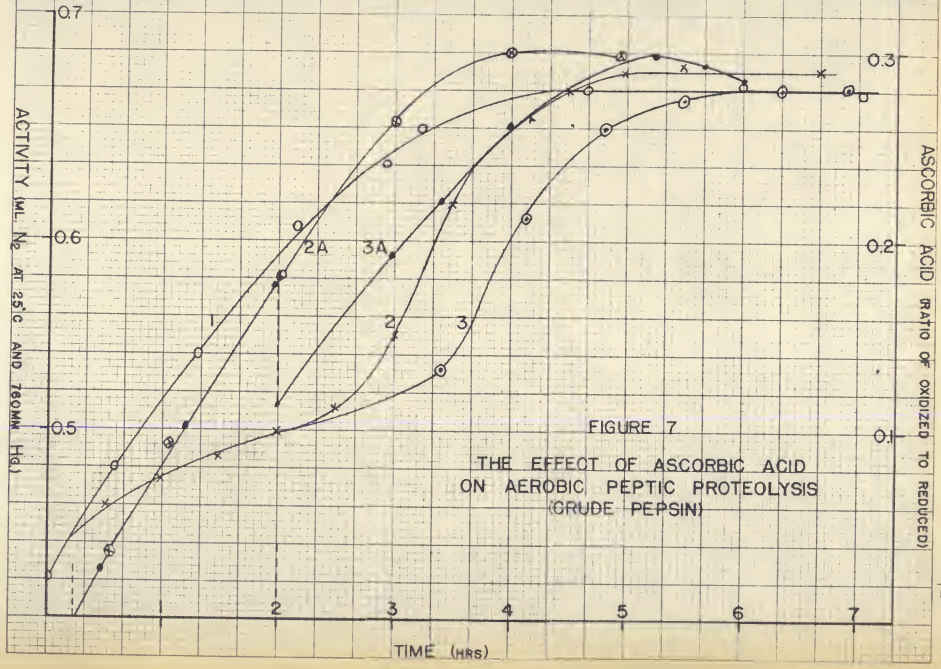

FIGURE 7

THE EFFECT OF ASCORBIC ACID
ON AEROBIC PEPTIC PROTEOLYSIS
(CRUDE PEPSIN)

ascorbic acid, observed during the course of digestion, increased gradully as shown in curve 2A, figure 1. At a point corresponding to the change in proteolytic rate (approximately 2½ hours) the ratio is 0.225. Similarly, curve 3A gives the ratio corresponding to digestion 3. The ratio of oxidized to reduced ascorbic acid at the inflection of proteolytic rate is 0.221. The agreement of these ratios suggested that the observed change in proteolytic rate was related to the oxidation of ascorbic acid.

Under anaerobic conditions, in the absence of ascorbic acid, no change in proteolysis occurs, figure 8, curve 4. In the presence of ascorbic acid, however, the rate of anaerobic proteolysis was decreased and furthermore showed no tendency towards acceleration even after 5½ hours, curve 5. The ratio of oxidized to reduced ascorbic acid, curve 5A, remained rather constant at about 0.1, well below the aerobic value of 0.225. The data suggest that an oxidized/reduced ratio of ascorbic acid of approximately 0.225 in a crude pepsin digestion leads to a loss of peptic inhibitory influence. Under anaerobic conditions oxidation is sufficiently retarded so that the critical ratio is not reached and thus peptic inhibition seems to continue. Although the mammalian stomach might be expected to contain some air, one would expect reduced ascorbic acid to be secreted rather constantly. The net effect would probably be to keep the ratio well below 0.225.

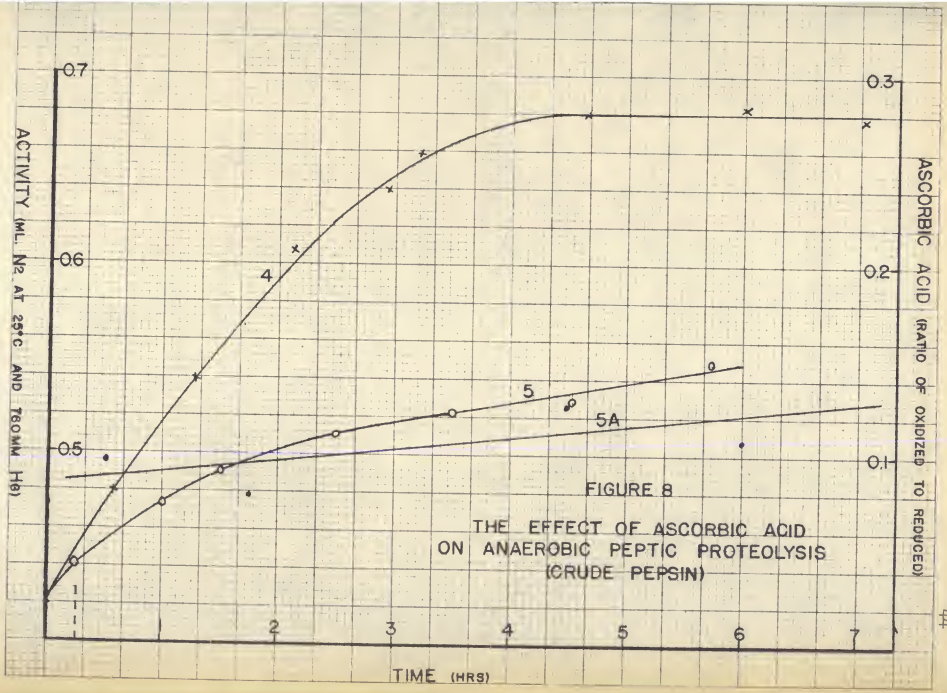

FIGURE 8

THE EFFECT OF ASCORBIC ACID
ON ANAEROBIC PEPTIC PROTEOLYSIS
(CRUDE PEPSIN)

In digestions 1-5, figures 7 and 8, larger than accepted physiological concentrations were employed to facilitate the detection of otherwise inconspicuous effects. The literature(50)(51) reports ascorbic acid concentrations of 1-2 mg. % for histamine-stimulated hydrochloric acid secretion, but it seems reasonable to suppose that secretion continues throughout digestion. To simulate the reported physiological conditions, aerobic proteolysis was studied using lower ascorbic acid and pepsin concentrations. The reduction in crude pepsin concentration resulted in slower hydrolysis of the casein confirming suspicions that this was a true enzymatic reaction, figure 9, curve 6. Addition of 5 mg. % ascorbic acid resulted in a rate decrease similar to those previously observed, figure 9, curve 7. The ratio, shown in curve 7A, increased during proteolysis and is 0.22 at the point of inflection. The total ascorbic acid, curve 7B, had essentially disappeared at 30 hours.

2. Crystalline pepsin experiments. The control digestions of casein with crystalline pepsin are shown in figure 10. Pepsin recrystallized from crude commercial material, curve 8, compared very favorably with that obtained commercially, curve 9, both in activity and under microscopic examination in polarized light. The addition of ascorbic acid to the crystalline pepsin digestion did not result in any peptic inhibition, as seen in curve 10.

FIGURE 9.
THE EFFECT OF ASCORBIC ACID ON PEPTIC PROTEOLYSIS (REDUCED QUANTITIES OF CRUDE PEPSIN AND ASCORBIC ACID UNDER AEROBIC CONDITIONS)

The data show that ascorbic acid exerts an inhibitory influence on peptic proteolysis when crude pepsin is employed. This inhibition is lost when the ascorbic acid ratio has risen to 0.225. Purification of the pepsin also results in a loss of ability to respond to the inhibitory effect of ascorbic acid. Some intermediary substance, extracted simultaneously from the mucosa in the preparation of pepsin, but lost in further purification, is involved in the inhibition. The nature of this substance is unknown. The question arose as to whether the substance acted by inhibiting the oxidation of ascorbic acid. A comparison of curve 7A, figure 9, which illustrates the oxidation of ascorbic acid in the presence of crude pepsin, with curve 11A, figure 11, which traces the oxidation in the presence of the crystalline enzyme, does not indicate any appreciable difference in the rate of oxidation. The exclusion of air from the digestion, curve 12, does result in an inhibition of the oxidation, curve 12A. Even in the presence of the low oxidized/reduced ratio there was no apparent inhibition of the digestion in the absence of the factor contained in the crude pepsin. This unknown substance may act by accepting hydrogen from ascorbic acid and thus exert its inhibitory effect in a reduced form. Oxidation of the unknown substance should convert it to its inert form. In the aerobic *in vitro* experiments using crude pepsin, the inhibitory effect is only transitory, but in the absence of air the effect is

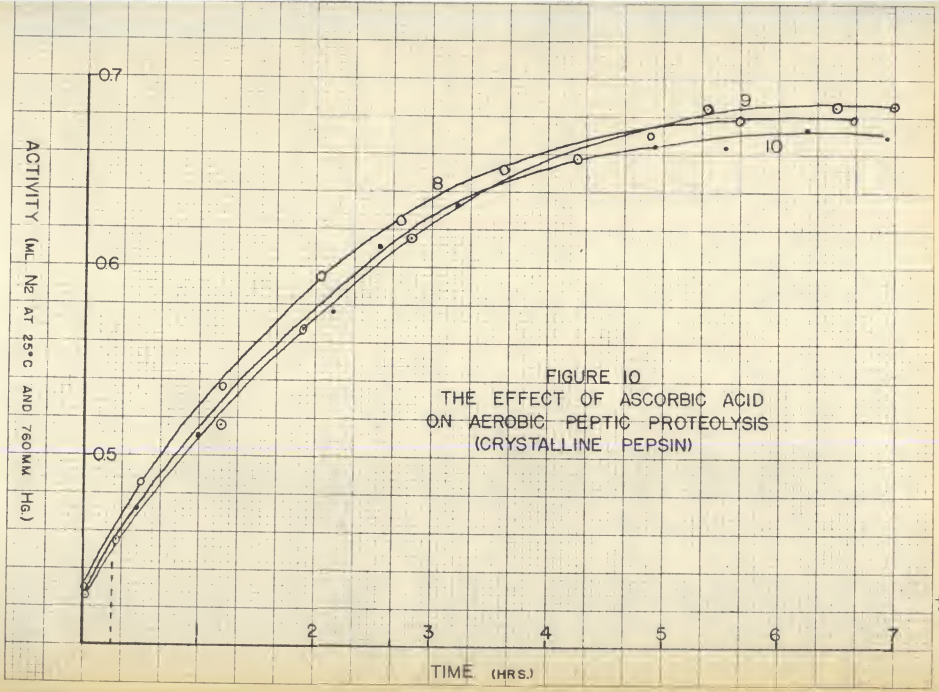

FIGURE 10
THE EFFECT OF ASCORBIC ACID
ON AEROBIC PEPTIC PROTEOLYSIS
(CRYSTALLINE PEPSIN)

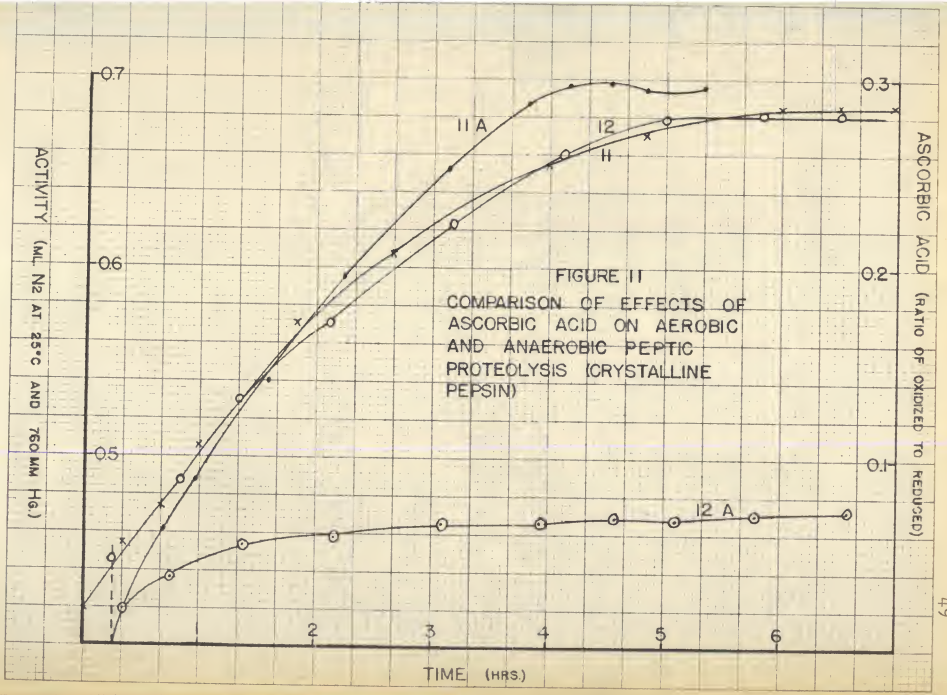

FIGURE 11
COMPARISON OF EFFECTS OF ASCORBIC ACID ON AEROBIC AND ANAEROBIC PEPTIC PROTEOLYSIS (CRYSTALLINE PEPSIN)

sustained. In vivo the inhibition should also be sustained since the supply of reduced ascorbic acid is probably relatively constant.

Recently, the results of a clinical study were reported which substantiate the data reported herein. The wound healing properties of ascorbic acid led Nash(86) to administer vitamin C together with an antacid to one group of ulcer patients, while administering only the antacid to another group. He concluded that although ascorbic acid apparently did not reduce the healing time of the ulcers, it did reduce the number of symptomatic relapses. It has been claimed that the symptomatic pain experienced by ulcer patients is correlated with the degree of acidity(87) of the gastric juice. In Nash's experiments, however, the decrease in relapses did not seem related to the acidity, since an antacid was administered to both groups. Friedman (88) has reported that the intake of food decreased the distress caused by peptic ulcers in 42 of 46 cases, presumably through the absorption of the acid gastric juice. As digestion proceeds, and the stomach empties, in many cases the symptomatic pain returns.

In the light of the data obtained in the present experiments, a hypothesis which explains Nash's results is that orally administered ascorbic acid retards gastric digestion, thus prolonging gastric retention of food. The slowly digesting food mass, absorbing gastric secretion,

reduces the duration of exposure of the ulcer to undiluted hydrochloric acid.

B. The Investigation of Possible Gastric Synthesis of Ascorbic Acid in the Rat.

To determine if there was a gastric synthesis of ascorbic acid, excised stomachs were suspended in Ringer-Tyrode solution and the ascorbic acid in the solution determined periodically. The stomachs continued to exhibit a visible peristaltic rhythm for longer than 100 minutes in each experiment. In experiments 1 and 2, Table 3, the empty

TABLE 3

Failure of the Rat Stomach to Synthesize Ascorbic Acid in vitro in Ringer-Tyrode at 37° C.

EXP. No.	MICROGRAMS TOTAL ASCORBIC ACID IN SUSTAINING SOLUTION AFTER INCUBATION OF STOMACH FOR					AVERAGE PERISTALTIC RATE (contr/min)
	1 min	30 min	60 min	90 min	360 min	
1*	84	84	83	85	-	5.2
2**	77	76	76	77	-	5.6
3***	79	79	79	77	70	19.1

* animal fasted 48 hours, sacrificed, stomach cavity rinsed.

** animal received C-free diet for 48 hours previous to sacrifice, stomach cavity rinsed.

*** non-fasted animal sacrificed, stomach cavity rinsed and filled with glass beads.

stomach gave an average of 5.4 contractions per minute. There was, however, no observable increase in ascorbic acid in the sustaining solution over the period of the experiment. It seemed a possibility that a full, more rapidly contracting stomach might prove more active in biosynthesis. For this reason the stomach of a non-fasted rat was removed, rinsed, filled with glass beads and suspended, Experiment 3. In this case active peristalsis continued for 372 minutes, the rate of contraction averaging 19.1 per minute. Although the stomach exhibited rhythmic contractions for longer than 6 hours, there was no increase in ascorbic acid in the sustaining solution.

Glucose, a constituent of the sustaining solution employed in these experiments, has been shown to be a precursor for ascorbic acid synthesis in the intact rat(33)(34)(35). The data in Table 3 indicate that in the presence of glucose, ascorbic acid is not synthesized in vitro by the rat's stomach. It is not unreasonable to suspect that the stomach of the intact rat is, likewise, not a site of synthesis of ascorbic acid. The vitamin C present in the gastric juice of the rat, as well as gastric tissue, must originate elsewhere.

C. Ascorbic Acid in the Gastric Tissue of the Normal and Gastritic Rat.

In this section, the effects of a eugenol-induced gastritis on the gastric and adrenal ascorbic acid were studied.

The results appear in Table 4. The reduced form of ascorbic

TABLE 4.

Effect of Eugenol Administration on Gastric and Adrenal Ascorbic Acid in Rats.

Group*	Number of rats**	Chemical form	Ascorbic Acid Concentration STOMACH glandular mg%	glandular γ/g	forestom. mg%	forestom. γ/g	ADRENALS mg%
1	10	ox	2.2	24	3.3	11	15.8
		red	22.2	244	11.6	38	364.8
		tot	24.4•	268	14.9•	49	380.6•
2	7	ox	2.9	32	2.7	9	16.6
		red	16.6	182	16.3	53	261.9
		tot	19.5	214	19.0	62	278.5
3	5	tot	24.8•	281	15.1•	50	266.6
4	5	tot	19.4	213	18.8	62	290.4

* Group 1 received water by stomach tube; group 2, eugenol emulsion by stomach tube; group 3, eugenol emulsion by duodenal injection; group 4, eugenol emulsion by gastric injection.

** Weight loss was not observed in any animal in these groups.

• Comparisons of corresponding totals from groups 1 and 2 and comparison of totals from groups 3 and 4 are statistically significant(89). P is less than 0.01 in each instance.

acid was found to be predominant in the stomach, as it was in the adrenal, which was studied concurrently as a reference organ. In the water-fed controls, Group 1, the reduced ascorbic acid accounted for 91% of the total ascorbic acid found in the glandular stomach, 78% of that found in the forestomach, and 96% of that found in the adrenals. The concentration of total ascorbic acid was significantly higher in the glandular stomach than in the forestomach. The glandular stomach of rats and mice is apparently less susceptible to spontaneous(76) and induced(77) dyscrasia than the forestomach. The possibility of a relationship between the pathological susceptibility of gastric tissue and the amount of ascorbic acid in the tissue should be considered.

Introduction of the eugenol emulsion to the gastric lumen by stomach tube, Group 2, resulted in a grossly recognizable gastritis which was distinctly absent in the water-fed controls. The total ascorbic acid concentration in the glandular stomach was significantly decreased in the gastritic rats, Group 2, while in the forestomach the concentration was significantly increased. The total amount of ascorbic acid in the complete stomach of the gastritic group was 13% less than that in the water-fed controls. The ratios of oxidized to reduced ascorbic acid found in the forestomach, glandular stomach, and adrenals of the gastritic group was not significantly different from those found in

comparable tissues of the water-fed controls.

Administration of eugenol emulsion by stomach tube, Group 2, resulted in a significant decrease in adrenal ascorbic acid. This reduction is suggestive of a stress phenomena occurring after intestinal absorption. The possibility that the decrease of ascorbic acid in the stomach could also have been a systemic effect made it advisable to place the eugenol directly in the duodenum. If the gastric effect observed in Group 2 was systemic in nature, the ascorbic acid in the stomach should be decreased. Injection of eugenol into the duodenal lumen, Group 3, did not evoke a grossly recognizable gastritis and the concentrations of ascorbic acid found in the fore- and glandular stomachs were almost identical to those of the water fed controls. Injection of eugenol into the duodenum avoided direct gastric mucosal contact with the eugenol, but allowed intestinal absorption as evidenced by the decrease in the adrenals. Since the adrenal decrease also occurred in the absence of operative procedure and anesthesia in Group 2, the reduction is probably related to the systemic presence of eugenol.

The injection of eugenol emulsion into the gastric lumen, Group 4, resulted in grossly recognizable gastritis and changes in gastric ascorbic acid concentration practically identical to those observed in the eugenol-fed, gastritic group, but significantly different from those in the duodenum-injected group. It may be concluded that the changes in the

gastric ascorbic acid during a eugenol-induced gastritis, as well as the gastritis itself, are associated with the direct contact of the eugenol with the gastric mucosa, and not a systemic effect after gastrointestinal absorption.

D. Ascorbic Acid in the Gastric Tissue of the Normal and Gastritic Guinea Pig.

It can be concluded from the previous section, IV C, that a eugenol-induced gastritis is associated with a localized gastric deficiency in ascorbic acid in the rat, which synthesizes ascorbic acid. It seemed pertinent, therefore, to investigate this effect in an animal which has a vitamin C requirement similar to man. The effects of a eugenol-induced gastritis on gastric and adrenal ascorbic acid were studied in the guinea pig. The results appear in Table 5.

TABLE 5

Effect of Eugenol Administration on Gastric and Adrenal Ascorbic Acid in Guinea Pigs.

Group	Number of G. Pigs	Treatment	Chemical Form	Ascorbic Acid Concentration STOMACH mg%	ADRENALS mg%
1	10	eug.	ox	1.0	3.4
			red	7.9	84.4
			tot	8.9•	87.8•
2	11	water	ox	1.5	5.3
			red	14.4	117.7
			tot	15.9	123.0

• Comparisons of corresponding totals from groups 1 and 2 are statistically significant(89). P is less than 0.01 in each instance.

The reduced form of ascorbic acid was found to be predominant in the stomach and adrenals. In the water-fed controls, Group 2, the reduced ascorbic acid accounted for 91% of the total ascorbic acid found in the stomach and 96% of that found in the adrenals.

Introduction of eugenol emulsion into the gastric lumen by stomach tube, Group 1, resulted in a grossly recognizable gastritis which was distinctly absent in the water-fed controls. The total ascorbic acid concentration in the stomachs of the gastritic guinea pigs was significantly decreased by 44.1%. In the same animals there was a simultaneous decrease of 28.6% in the adrenals. The ratio of oxidized to reduced ascorbic acid in the stomachs and adrenals of the gastritic animals was not different from that in the controls.

The trend of the results is strikingly similar to those found in an identical experiment with rats, section IV C. In the light of these experiments, it can be concluded that the induction of gastritis with eugenol is associated with a decrease in gastric ascorbic acid. A decrease in adrenal ascorbic acid occurs simultaneously. The decrease in the stomach tissue, as well as the gastritis itself, is associated with the direct contact of the irritant with the mucosa, while the decrease in the adrenal is probably a manifestation of stress.

E. Relationships Between Ascorbic Acid and Gastric Secretion.

In order to determine if a mild deficiency of ascorbic acid would influence gastric secretion, gastric juice was collected from prescorbutic(Group 1) and non-scorbutic(Group 2) guinea pigs, each having a gastro-cutaneous fistula. Each animal designated as prescorbutic had received a low complement of ascorbic acid for 14 days or more and exhibited the typical shaggy appearance together with a rather static body weight. The volume of gastric juice collected per unit time was recorded and the normality and ascorbic acid concentration determined. The effects of saline, ascorbic acid and histamine, injected separately during the collection periods, were studied. The results appear in graphic form in Figures 12 through 15. The individual points on each curve represent the average of not less than 16 determinations; analyses of 4 collections of gastric juice from each of 4 guinea pigs.

The normality, volume, and ascorbic acid concentration in the gastric secretion of non-scorbutic guinea pigs are depicted in Figure 12. Curve A follows the normality of the gastric juice throughout the 75 minute collection period. At the start of the collection, the normality of the latent gastric juice, accumulated during the precollection fasting period, was 0.140. After rinsing the gastric lumen with saline, the normality fell to 0.018, but increased rapidly and became constant at approximately 0.100. The rate

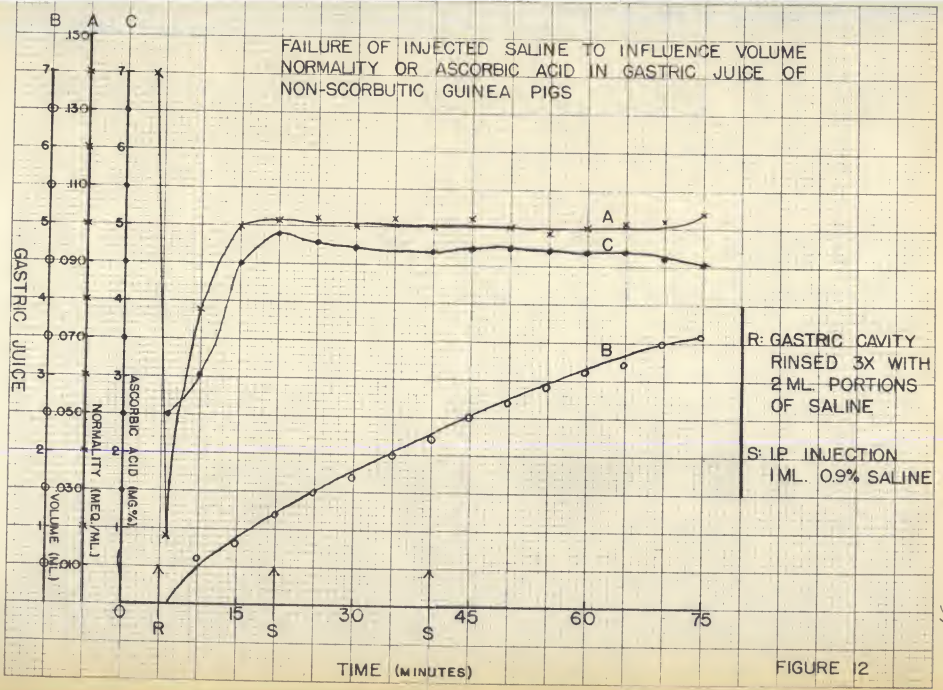
FAILURE OF INJECTED SALINE TO INFLUENCE VOLUME NORMALITY OR ASCORBIC ACID IN GASTRIC JUICE OF NON-SCORBUTIC GUINEA PIGS
B
A
C
GASTRIC JUICE
VOLUME (ML.)
NORMALITY (MEQ./ML.)
ASCORBIC ACID (MG%)
A
C
B
R: GASTRIC CAVITY RINSED 3X WITH 2 ML. PORTIONS OF SALINE
S: I.P. INJECTION 1 ML. 0.9% SALINE
R
S
S
TIME (MINUTES)

FIGURE 12

of secretion of gastric juice, shown in curve B, continued rather constant. The ascorbic acid concentration increased rapidly in the freshly collected juice and became constant at 4.5-5.0 mg%. It is perhaps surprising to note that this range is much higher than previously reported for human and canine gastric juice(50)(51). Reasons for this apparent discrepancy will be discussed later in this section. The intraperitoneal injection of saline did not influence the normality, rate of secretion, or ascorbic acid concentration of the gastric juice.

Identical experiments were performed with prescorbutic animals. The normality of the gastric juice in the prescorbutic animals, curve A, Figure 13, became constant at 0.080, which represents a 20% decrease in hydrochloric acid concentration when compared with the non-scorbutic group of guinea pigs. The rate of secretion of gastric juice, curve B, was not, however, different from that of non-scorbutic controls. This decreased concentration of hydrochloric acid in the secreted gastric juice together with an unchanged rate of secretion suggests two possible mechanisms. First, the hydrochloric acid elaborated by the parietal cells in the stomachs of the prescorbutic group may have been of decreased normality. This hypothesis is, however, in disagreement with the theories of Pavlov(90) and Hollander(91)(92), who believe that the parietal cell elaborates hydrochloric acid of a constant normality. The

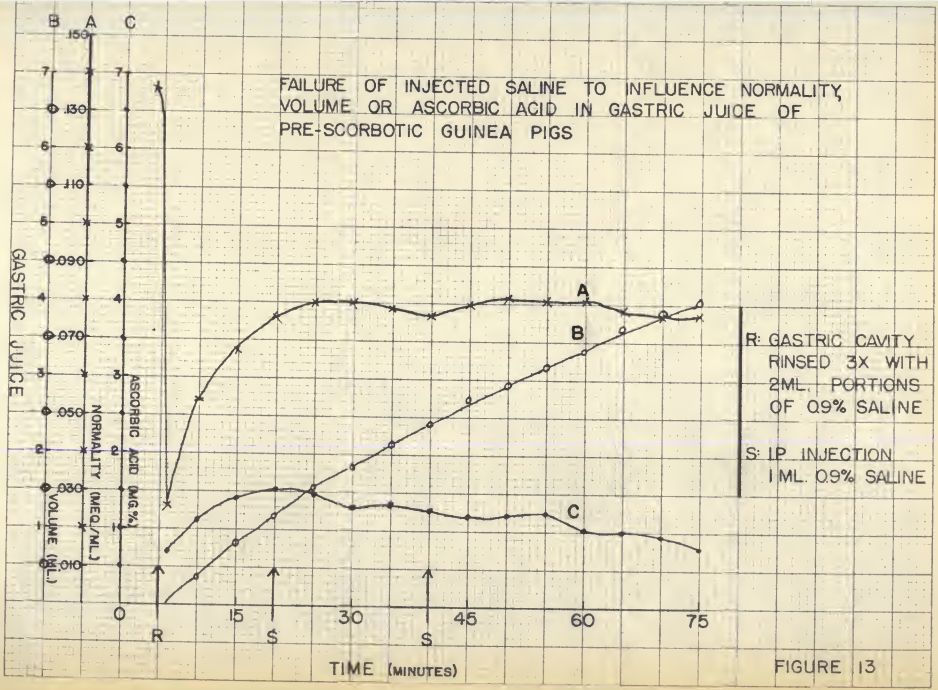
FAILURE OF INJECTED SALINE TO INFLUENCE NORMALITY, VOLUME OR ASCORBIC ACID IN GASTRIC JUICE OF PRE-SCORBOTIC GUINEA PIGS
B
A
C
GASTRIC JUICE
VOLUME (ML.)
NORMALITY (MEQ./ML.)
ASCORBIC ACID (MG.%)
.150
.130
.110
.090
.070
.050
.030
.010
7
6
5
4
3
2
1
0
A
B
C
R: GASTRIC CAVITY RINSED 3X WITH 2ML. PORTIONS OF 0.9% SALINE
S: I.P. INJECTION 1 ML. 0.9% SALINE
R
S
S
0
15
30
45
60
75
TIME (MINUTES)

FIGURE 13

alternative explanation is that only the rate of secretion of hydrochloric acid was diminished in the prescorbutic animals, while the rate of secretion of mucosal components, other than parietal, was correspondingly increased.

The ascorbic acid concentration in the gastric juice, curve C, decreased to a concentration of 1.0-1.5 mg% in the prescorbutic animals. This represents at least a 66% decrease in the concentration when compared with the non-scorbutic controls. It is not surprising that the decreased intake of ascorbic acid in these animals should be reflected by a decreased concentration of the vitamin in the gastric juice.

The injection of saline did not influence the normality, volume or ascorbic acid concentration of the gastric juice. Since the intraperitoneal injection of saline had not altered the character of gastric secretion in non-scorbutic or prescorbutic animals, it was then used as a vehicle for the injection of ascorbic acid and histamine.

The results of the injection of ascorbic acid and the injection of histamine observed in the gastric secretion of non-scorbutic guinea pigs appear in Figure 14. After the normality, curve A, had risen to its previously determined level of approximately 0.100, ascorbic acid was injected. At 25 minutes, 5 minutes after the vitamin was injected, the normality of the gastric juice increased sharply and

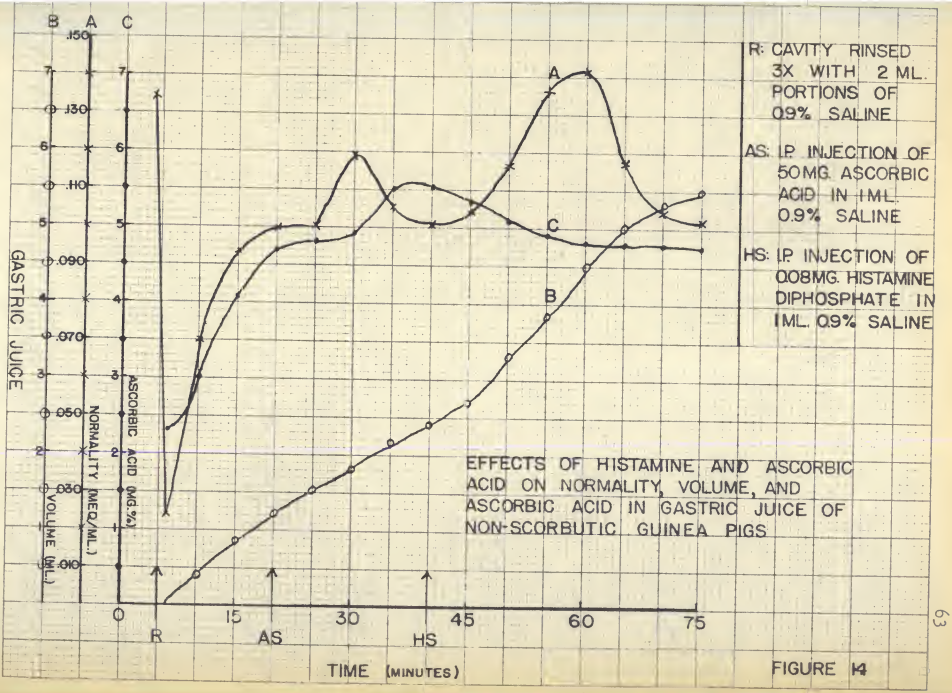
B
A
C
GASTRIC JUICE
VOLUME (ML.)
NORMALITY (MEQ/ML.)
ASCORBIC ACID (MG.%)
.150
.130
.110
.090
.070
.050
.030
.010
7
6
5
4
3
2
1
0
A
B
C
R
AS
HS
0
15
30
45
60
75
TIME (MINUTES)
R: CAVITY RINSED 3X WITH 2 ML. PORTIONS OF 0.9% SALINE
AS: I.P. INJECTION OF 50 MG. ASCORBIC ACID IN 1 ML. 0.9% SALINE
HS: I.P. INJECTION OF 0.08 MG. HISTAMINE DIPHOSPHATE IN 1 ML. 0.9% SALINE
EFFECTS OF HISTAMINE AND ASCORBIC ACID ON NORMALITY, VOLUME, AND ASCORBIC ACID IN GASTRIC JUICE OF NON-SCORBUTIC GUINEA PIGS

FIGURE 14

reached a peak of 0.119 at 30 minutes. This increase represents a rise of approximately 20% in the concentration of hydrochloric acid over the non-scorbutic control value of 0.100 N. A sharp decrease followed which reduced the normality to the control level. This momentary rise and fall in normality was not accompanied by any change in the rate of secretion, curve B. Once again, one must conclude that either the parietal cells can secrete hydrochloric acid of varying normality or that an increased rate of secretion by these cells is accompanied by a decreased rate of secretion by other gastric components. The injection of histamine elicited a response which was not unexpected, curves A and B. The normality of the gastric juice increased sharply, attaining a peak value of 0.141, then falling off toward the control level. The rate of secretion increased concurrently with the increase in normality, suggesting that only the parietal cells were being stimulated, secreting hydrochloric acid at an increased rate.

The ascorbic acid concentration in the gastric juice, curve C, reached a control level of approximately 4.8 mg%. Shortly after the injection of the ascorbic acid this value rose to 5.5 mg%. The concentration then slowly decreased and was decreasing concurrently with the increase in normality and rate of secretion previously discussed. The injection of histamine apparently did not stimulate the secretion of ascorbic acid in the gastric juice. It is

probable that determinations of ascorbic acid in gastric juice of histamine stimulated individuals may incorporate a sizeable negative error caused by simple dilution of the existing ascorbic acid with the freshly secreted hydrochloric acid.

The results of the injection of ascorbic acid and histamine to prescorbutic guinea pigs are presented in Figure 15. The normality of the collected gastric juice, curve A, increased and became constant at approximately 0.080, confirming the results obtained earlier in the same animals. The injection of ascorbic acid brought about an elevation of the normality to 0.099. The injection of histamine elicited another increase in normality which attained a peak at 0.138. The corresponding increase in normality in the non-scorbutic animals exhibited a maximum 0.141, curve A, Figure 14. The agreement of the two values indicates that prescorbutic and non-scorbutic guinea pigs respond with similar increases in the normality of gastric juice. These findings confirm those of Nordström(93) who showed that the histamine response in scorbutic guinea pigs was either the same or slightly lower than in non-scorbutic animals. The rise in normality associated with the injection of ascorbic acid to prescorbutic animals was not accompanied by an increase in the rate of secretion, curve B, Figure 15, while the increase in normality caused by histamine was associated with a corresponding increase in the rate of

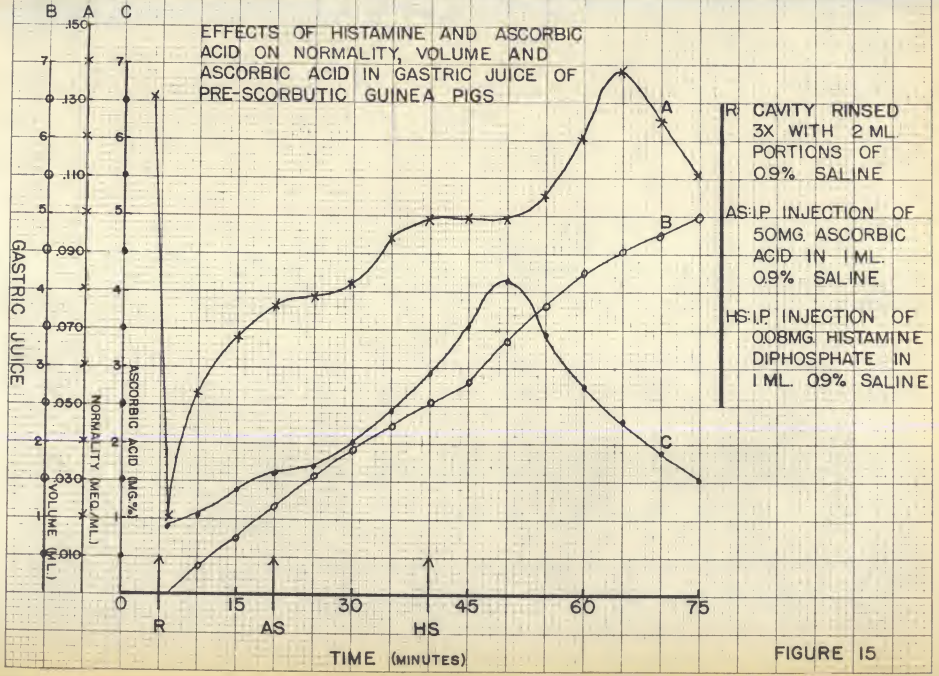
EFFECTS OF HISTAMINE AND ASCORBIC ACID ON NORMALITY, VOLUME AND ASCORBIC ACID IN GASTRIC JUICE OF PRE-SCORBUTIC GUINEA PIGS
B
A
C
GASTRIC JUICE
VOLUME (M.L.)
NORMALITY (MEQ./ML.)
ASCORBIC ACID (MG.%)
.150
.130
.110
.090
.070
.050
.030
.010
7
6
5
4
3
2
1
0
15
30
45
60
75
R
AS
HS
TIME (MINUTES)
A
B
C
R: CAVITY RINSED 3X WITH 2 ML. PORTIONS OF 0.9% SALINE
AS: I.P. INJECTION OF 50MG. ASCORBIC ACID IN 1 ML. 0.9% SALINE
HS: I.P. INJECTION OF 0.08MG. HISTAMINE DIPHOSPHATE IN 1 ML. 0.9% SALINE

FIGURE 15

secretion.

The concentration of ascorbic acid in the gastric juice of the prescorbutic guinea pigs, curve C, was again found to range from 0.9-1.6 mg%. The injection of ascorbic acid caused a dramatic increase in the concentration of the vitamin in the gastric juice which reached a maximum of more than 4 mg%. The concentration soon began to decrease, however, and was decreasing during the previously described increase in normality and rate of secretion. This is added evidence that histamine does not stimulate the secretion of ascorbic acid in the gastric juice.

It can be stated from the results of these experiments that (a) the normality of guinea pig gastric juice is approximately 20% lower during a mild ascorbic acid deficiency than during the administration of adequate ascorbic acid.

(b) ascorbic acid is secreted in the gastric juice of guinea pigs but such secretion is not stimulated by histamine.

(c) the injection of ascorbic acid to prescorbutic or non-scorbutic guinea pigs results in a slight increase in the normality of secreted gastric juice, which is not accompanied by a simultaneous increase in volume.

F. A Study of Dietary Administration of the Carcinogen, 2-Aminofluorene, to Guinea Pigs Receiving High and Low Doses of Ascorbic Acid.

Four groups of 12 guinea pigs were employed in these experiments. All were allowed Purina Rabbit Chow Checkers (PRCC) or Purina Rabbit Chow Checkers impregnated with 0.06% 2-aminofluorene(PRCC-AF) and water ad libitum. Group 1 received PRCC and an elevated dose of ascorbic acid; group 2 received PRCC-AF and the same elevated dose of ascorbic acid. Group 3 received PRCC and a low dose of ascorbic acid; group 4 received PRCC-AF and the same low dose of ascorbic acid (see Table 2). The animals consumed an average of 35 g. of food per day which is equivalent to a daily intake of approximately 21 mg. aminofluorene in groups 2 and 4. The animals receiving the larger ration of ascorbic acid, groups 1 and 2, gained weight rapidly, while those receiving the smaller amount, groups 3 and 4, gained rather slowly. The animals were kept on this regimen for 267 days after which they were fed the stock guinea pig diet of this laboratory, Purina Rabbit Chow Checkers, water, and fresh lettuce ad libitum. At the time the administration of the carcinogen was discontinued there were only 25 animals alive. The most prominent cause of death was a respiratory infection which was grossly similar to bronchopneumonia. No tumors were found at autopsy of these animals. No palpable tumors had appeared in the remaining animals by

the 297th day.

It may be significant that no tumors were induced in the guinea pig under these conditions, since Morris et al (94) have shown that similar administration of aminofluorene to rats resulted in a high incidence of tumors. Table 6 compares the results of Morris with those observed in the present investigation. It must be pointed out that the periods of observation in these experiments were similar, but the total intake of aminofluorene was approximately 10 times higher in the guinea pig than in the rat.

TABLE 6

Comparison of the Carcinogenicity of 2-Aminofluorene in the Rat and Guinea Pig

SPECIES	ORAL ADMINISTRATION OF AF*				OBSERVED (days)	TUMORS•
	% in diet	mg per day	length of adm. (days)	total mg		
G. Pig	0.06	21	267	5600	297	0/12
Rat**	0.05	3.3	161	537	284	9/11

* aminofluorene, m.p. 126-127.5

• the numerator indicates the total number of tumors; the denominator, the total number of animals.

** The results given for the rat are based on those reported by Morris et al(94).

It can be stated that in comparable periods of administration and observation, aminofluorene induces tumors in the rat, but does not in the guinea pig. Because no tumors were produced

in any group of guinea pigs, the effect of reduced ascorbic acid administration, of course, cannot be evaluated.

V. CONCLUSIONS

This study of ascorbic acid in its bearing on gastric physiology and pathology has established the presence of several relationships which may well assume clinical significance.

A. Gastric Secretion.

The guinea pig with a surgically prepared gastrocutaneous fistula may be employed very satisfactorily in the study of gastric physiology. By employing this technique, it has been demonstrated that the secretion of ascorbic acid into the gastric cavity is not stimulated by histamine. Therefore, a more accurate value for ascorbic acid concentration in gastric secretion will be obtained by analyzing the gastric juice of subjects not stimulated by histamine, since in this manner, the ascorbic acid will not be diluted by accumulating hydrochloric acid. The normality of the gastric secretion of prescorbutic and non-scorbutic guinea pigs was essentially the same after stimulation with histamine, however, a decreased intake of ascorbic acid, simulating a subclinical deficiency, in the absence of a secretory stimulant, resulted in a decrease in the hydrogen-ion concentration of gastric secretion. The injection of ascorbic acid brought about recovery to the physiologic level. These findings imply that ascorbic acid deficiencies of the subclinical

variety may result in the impairment of gastric digestive efficiency.

B. Gastric Digestion.

The digestion of a common protein with crude pepsin is retarded by ascorbic acid. Aerobic oxidation of the ascorbic acid to a particular oxidized/reduced ratio results in an increase in the rate of proteolysis. Digestions using crystalline pepsin are not so affected by ascorbic acid. This leads to the postulation that some other substance, extracted from hog and bovine mucosa in the preparation of crude pepsin, is the actual inhibitor and is activated by reduced ascorbic acid. This postulated substance may also be a constituent of human gastric juice. In this case, the oral administration of ascorbic acid might be indicated in cases of gastric ulcer, where many times it is desirable to bring about a retardation of the digestive processes in the stomach.

C. Gastritis.

A gastritis induced by direct contact of eugenol with the gastric mucosa is characterized by a decrease in gastric tissue ascorbic acid. In the guinea pig this reduction amounts to approximately 44%. In rats, a species which synthesizes ascorbic acid, the reduction in the stomach tissue is about 13%. It is well to mention here that in an in vitro study of the stomach of the rat, synthesis of ascorbic acid by this organ could not be demonstrated. Therefore,

the ascorbic acid in the gastric juice of the rat, as well as that in the gastric tissue, originates elsewhere. The decrease in gastric tissue ascorbic acid suggests that vitamin C is utilized rapidly enough in the regenerative processes operating to replace desquamated epithelial tissue, so that a localized deficiency develops in spite of an efficient biosynthesis in the case of the rat. It may well be that the oral administration of ascorbic acid in cases of acute and chronic gastritis will prove beneficial in the repair of the mucosa.

D. Tumor Induction.

Although the effect of ascorbic acid on tumor induction in guinea pigs cannot be evaluated, this study has made possible a comparison of the carcinogenicity of 2-aminofluorene in two species under similar experimental conditions. The oral administration of 2-aminofluorene to rats has been reported by other workers to induce a high percentage of tumors, Table 6, while in this investigation, no tumors were found after similar administration of the same compound to guinea pigs. These results strongly suggest that the metabolism of the compound is different in the two species. A study of the metabolism of 2-aminofluorene in the guinea pig, comparable to that already completed in this laboratory for the rat, should prove helpful in understanding the resistance of the guinea pig to chemical carcinogenesis----possibly an important phenomena to be con-

sidered in the ultimate control of cancer.

VI. SUMMARY

The effect of ascorbic acid deficiency on gastric secretion has been investigated. Studies of the gastric juice of fistulate guinea pigs, suffering a subclinical ascorbic acid deficiency, indicate that the normality of the gastric secretion is approximately 20% lower than in guinea pigs receiving adequate vitamin C; injection of ascorbic acid results in recovery to the physiologic level; ascorbic acid secreted in the gastric juice is not stimulated by histamine.

The effect of ascorbic acid on gastric digestion of protein has been investigated *in vitro*. Casein was incubated aerobically and anaerobically at 37° C. using crude or purified pepsin preparations in hydrochloric acid at pH 2. Ascorbic acid was added to some digestions. Amino nitrogen and ascorbic acid analyses indicate that ascorbic acid retards peptic proteolysis when crude pepsin is employed, and that oxidation of the vitamin to a point where the oxidized to reduced ratio reached 0.225 results in a loss of peptic inhibitory influence. No inhibitory effect was observed when crystalline pepsin was used. The presence of another substance in the crude pepsin, the actual inhibitor, and its mechanism of action in relation to the vitamin, is postulated.

The results of a study of gastric tissue ascorbic acid in non-treated and gastritic animals have been reported. A gastritis was induced in rats and guinea pigs by the direct contact of an irritant, eugenol, with the gastric mucosa. Studies of the mucosa in normal and gastritic animals in each species reveal that the gastritis is characterized by a decrease in the concentration of ascorbic acid in stomach tissue.

The possibility of gastric synthesis of ascorbic acid in the rat was investigated. No synthesis of ascorbic acid by gastric tissue was evident.

Although administration of the carcinogen, 2-aminofluorene, did not induce tumors in guinea pigs receiving either a high or low intake of vitamin C, it was demonstrated that under similar experimental conditions the guinea pig is much more resistant to carcinogenesis than the rat. The clinical significance of this work is discussed.

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The author was born in Newark, New Jersey on June 20, 1924. He attended elementary and high schools in Irvington, Springfield and Maplewood, New Jersey. In November 1942 he enlisted in the United States Army, serving overseas with the 254th Infantry Medical Detachment. He is the holder of the purple heart, bronze star, Medical Valor Badge and Unit Presidential Citation. Upon his discharge in 1946, he married Jeanne Barbara Forsberg of Maplewood, New Jersey and is now the father of one daughter, Barbara Beth. He attended the University of Cincinnati receiving the B.S. degree in Chemistry and Zoology in 1949. He then accepted a fellowship at the Cancer Research Laboratory, University of Florida, where he received the M.S. degree in 1950. His major field of study has been cancer research.

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This dissertation was prepared under the direction of the candidate's Supervisory Committee and has been approved by all the members of the Committee. It was submitted to the Graduate Council and was approved as partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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